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Dauricine inhibited L-type calcium current in single cardiomyocyte of guinea pig[1]

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KEY WORDS dauricine; patch-clamp techniques; calcium channels; myocardium; cultured cells

AIM: To study the effect of dauricine (Dau) on L-type calcium current in guinea pig ventricular myocytes. **METHODS**: Using whole-cell recording method to record L-type calcium current (I_{Ca}) in single ventricular cell of guinea pig. **RESULTS**: Dau 1, 10, and 100 $\mu mol \cdot L^{-1}$ markedly reduced I_{Ca} by 15.2 % ± 2.2 %, 41 % ± 5 %, and 82 % ± 8 %, respectively. After washing out, I_{Ca} partially recovered. Dau inhibited I_{Ca} at 3 Hz and 1 Hz to a similar extent, its effect on I_{Ca} appeared to be not frequency-dependent. **CONCLUSSION**: Dau had a calcium channel blocking effect.

Dauricine [2-hydroxy-5,4′-bis(2-methyl-6,7-dimethoxy-1,2,3,4-tetra-hydroisoguinolin-1-ylmethyl) diphenyl ether], was isolated from rhizome of *Memispermum dauricum* DC. Its anti-arrhythmic effect had been demonstrated in various animals[1]. Our studies suggested that Dau play an importent role to efficient antagonism to delayed afterdepolarizations (DAD) and triggered activity (TA) by preventing an increase of myocardial cytoplasmic Ca^{2+} activity[2,3]. The present experiments were to reveal the effect of dauricine on L-type calcium current (I_{Ca}).

MATERIALS AND METHODS

Solutions and drugs The Ca^{2+}-free solution contained NaCl 100, KCl 10, KH_2PO_4 1.2, $MgSO_4$ 5, glucose 20, taurine 20, and 3-(*N*-morpholino) propanesulfonic acid (MOP) 10 $\mu mol \cdot L^{-1}$, pH 7.2 – 7.4 with KOH. The enzyme for cell dispersion was collagenase (type II, Sigma, final concentration 300 $kU \cdot L^{-1}$) in the nominally Ca^{2+}-free solution. All solutions used during the cell isolation procedure were oxygenated and maintained at 37 ℃.

The external solution used to superfuse cells during recording of currents contained NaCl 137, KCl 51.4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, HEPES 10, glucose 10, tetraethyl ammonium chloride (TEACl) 20 $mmol \cdot L^{-1}$, pH adjusted to 7.2 – 7.4 with NaOH at 24 – 28 ℃. The pipette solution contained CsCl 80, CsOH 40, $MgCl_2$ 2, egtazic acid 10, HEPES 10, Mg-ATP 5 $mmol \cdot L^{-1}$, pH adjusted to 7.2 – 7.4 with CsOH.

Dauricine supplied by Dr PAN Xi-Ping (Pharmaceutial College of Tongji Medical University), was a white powder, M_r 624, mp 103 – 104 ℃, purity > 99 %. It was dissolved in distilled water to 1 $mmol \cdot L^{-1}$.

Cell preparation Enzymatic dissociation method was used to prepare single ventricular cell[4,5]. Guinea pig (250 ± s 25 g, supplied by Experimental Animal Center, Shanghai Institute of Physiology) hearts were perfused retrogradely through the aorta at a rate of 10 $mL \cdot min^{-1}$ with oxygenated solution first with the standard Ca^{2+}-free solution for 5 min; then with the same solution containing collagenase 300 $kU \cdot L^{-1}$, 1 % bovine serum albumin (BSA), and $CaCl_2$ 50 $\mu mol \cdot L^{-1}$ for 5 min. The enzyme-digested hearts were cut until single cells were dissociated. The cells were filtered from 200 μm nylon mesh, resuspended in Ca^{2+}-free solution containing $CaCl_2$ 1.8 $mmol \cdot L^{-1}$, and stored at 24 – 26 ℃ for < 10 h.

Whole cell patch-clamp technique A small aliquot (0.1 mL) of dissociated cells was placed in a 0.5-mL chamber mounted on an inverted microscope (model CK2, Olympus). Cells were allowed to adhere to the coverslip and superfused at 24 – 28 ℃. Complete replacement of external solution 2 $mL \cdot min^{-1}$ in the chamber was achieved within 2 – 3 min.

The currents were recorded by the whole-cell configuration of the patch-clamp technique with a CEZ 2300 amplifier (Nihon Kohden). Voltage-clamp command was generated by a 12-bit digital-to-analog converter (model TL/1, Axon Instruments Inc) controlled by the pCLAMP Software Package (version 5.6, Axon Instrument Inc). Heat-polished patch-clamp pipette electrodes had a tip resistance of 3 – 5 MΩ when filled the pipette solution. Pipette capacitance and series resistance were compensated to minimize the duration of capacitive currents.

Protocol Rod-shape cells with clear cross-striations and resting potentials of at least – 78 mV were used. I_{Ca} was elicited in cells held at – 40 mV (to inactivate I_{Na}) and depolarized by pulses (40 mV) lasting 300 ms, a voltage-step (– 40 mV to + 60 mV) was used to obtain the current-voltage

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($I-V$) relation of I_{Ca}. To study the use-dependent effect of dauricine on I_{Ca}, I_{Ca} was elicited by a train of 20 depolarizing voltage step (250 ms in duration) from holding potential (V_h) of −40 mV to a test potential (V_t) of 0 mV delivered at a frequency of 1 or 3 Hz.

Data analysis Data were expressed as $\bar{x} \pm s$ and stored on hard disk for subsequent analysis with the pCLAMP software (version 5.6, Axon Instrument Inc). Statistical significance was determined by paired t-test.

RESULTS

Effect of dauricine on I_{Ca} When cell was held at −40 mV, and given a 40 mV, 300 ms 1 Hz depolarizing pulse, I_{Ca} reached its maximum. Under this condition the activities of Na^+ channel and T-type Ca^{2+} current were completely inhibited, and L-type Ca^{2+} current was measured. Five min after perfusion with external solution containing Dau 1, 10, and 100 $\mu mol \cdot L^{-1}$, the peak currents of I_{Ca} were reduced from 840 ± 97, 840 ± 96, 840 ± 93 pA in the absence of Dau to 710 ± 84 ($P<0.05$, $n=6$), 500 ± 61 ($P<0.01$, $n=6$), 150 ± 20 pA ($P<0.01$, $n=6$), respectively. Dau 1, 10, and 100 $\mu mol \cdot L^{-1}$ markedly reduced I_{Ca} by 15.2 % ± 2.2 %, 41 % ± 5 %, and 82 % ± 8 %, respectively. After washing out Dau with external solution, the peak of I_{Ca} partially returned (Fig 1). These results suggested that Dau inhibited I_{Ca}.

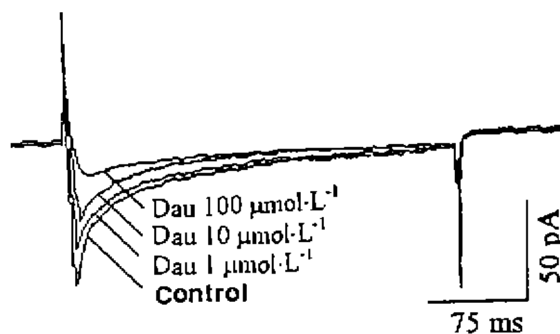

Fig 1. Effect of Dau on I_{Ca} of guinea pig ventricular single cell. Holding potential −40 mV; Command potential 40 mV; Stimulating duration 300 ms.

Effect of Dau on current-voltage relationship of I_{Ca} Cells were depolarized from a holding potential of −40 mV to +60 mV in steps of 10 mV resulting in an activation and inactivation of I_{Ca}. The amplitude of I_{Ca} before and after exposure to Dau 1, 10, and 100 $\mu mol \cdot L^{-1}$ showed lower than predrug level at membrane potential −20 mV to +60 mV. The maximal activation of I_{Ca} appeared at 0 mV in the presence of Dau (Fig 2).

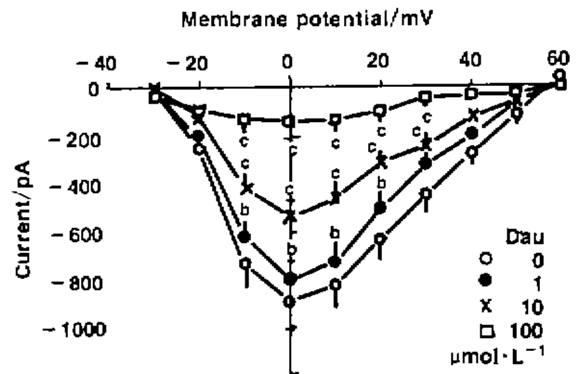

Fig 2. Superimposed current-voltage relations of I_{Ca} before and after exposure to Dau. $n=6$, $\bar{x} \pm s$. [b]$P<0.05$, [c]$P<0.01$ vs control.

Use-dependent effect of Dau on I_{Ca} I_{Ca} was elicited by a train of 20 depolarizing voltage steps from a V_h of −40 mV to a V_t 0 mV depolarizing in duration 250 ms at a frenquency of 1 or 3 Hz, Dau 10 $\mu mol \cdot L^{-1}$ inhibited I_{Ca} at 1 Hz and 3 Hz to a similar extent, appeared to be less frequency-dependent. Dau inhibited I_{Ca} in a normal use-dependent (not reverse) manner (Fig 3).

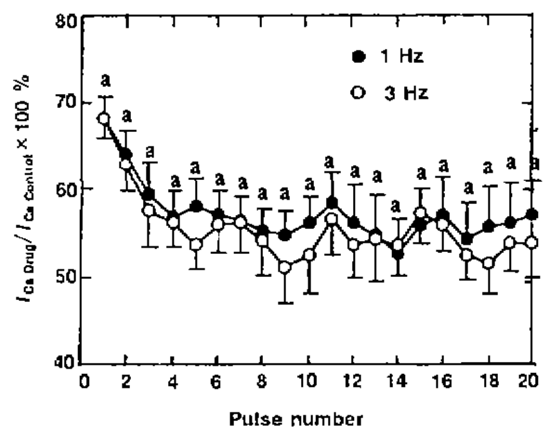

Fig 3. Frequency-independent block of I_{Ca} by Dau. Peak I_{Ca} was measured during each pluse under control conditions and Dau 10 $\mu mol \cdot L^{-1}$. The ratio of I_{Ca} after drug application to the control is shown. $n=5$ cells. [a]$P>0.05$ vs 3 Hz.

The inhibition during the last pulse of the train at

1 Hz was not significant than at 3 Hz (Tab 1).

Tab 1. Frequency-independent inhibition of I_{Ca} by Dau. $n = 5$ cells, $\bar{x} \pm s$. $^{a}P > 0.05$ vs 1 Hz.

I_{Ca}/pA		Control	Dau	Block %
Pulse 1	1 Hz	670 ± 128	480 ± 89	31 ± 3
	3 Hz	580 ± 95	400 ± 79	32 ± 4[a]
Pluse 20	1 Hz	760 ± 134	440 ± 78	42 ± 4
	3 Hz	590 ± 97	270 ± 45	44 ± 5[a]

DISCUSSION

Rundown of ionic currents is always a concern in whole-cell voltage-clamp recording. We minimized time-dependent changes in I_{Ca} by using high resistance pipettes filled with MgATP 5 $\mu mol \cdot L^{-1}$, and by performing the experiments ≤6 min after membrane rupture[6]. Moreover, a strict compare of timecouse of I_{Ca} was used in the experiment, in the normal control group ($n = 4$), after 5, 10, 15 min the I_{Ca} reduced only by 3.4 %, 14.7 %, 23.4 %, respectively. Comparatively, in the experimental group, Dau 2 and 10 $\mu mol \cdot L^{-1}$ for 10 min markedly reduced I_{Ca} by 28.4 %, 59.3 % respectively, and after washing out Dau, I_{Ca} were partially recovered, indicating the effect of Dau on I_{Ca} was not the consequence of the rundown of I_{Ca}.

Dau prolonged APD in a use-dependent manner in guinea pig papillary muscles[7]. In canine Purkinje fibers, Dau prolonged APD at fast rate and at slow rate to a similar extent, the effect of Dau on APD like amiodarone[8]. In this experiment, Dau appeared to be less frequency-dependent on inhibiting I_{Ca}. The results above also suggest that Dau block I_K in a use-dependent manner. We will further investigate the effect of Dau on I_K. Dau inhibited I_{Ca} in a concentration-dependent and frequency-independent manner (not reverse) suggesting that Dau might be promising in the treating of tachyarrhythmia.

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蝙蝠葛碱对豚鼠心室肌细胞 L 型钙电流阻断作用

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关键词 蝙蝠葛碱; 膜片箝技术; 钙通道; 心肌; 培养的细胞

目的: 研究蝙蝠葛碱对豚鼠心室肌细胞 L 型钙电流的阻断作用及其特性. **方法**: 利用全细胞记录方法, 记录单个豚鼠心室肌细胞 L 型钙电流. **结果**: 蝙蝠葛碱 1, 10, 100 $\mu mol \cdot L^{-1}$ 可使钙电流分别减少 15.2 % ± 2.2 %, 41 % ± 5 %, 82 % ± 8 %. 冲洗后, 可使钙电流部分恢复, 蝙蝠葛碱具有浓度依赖性阻断钙电流的作用. 在刺激频率 3 Hz 与 1 Hz, 其阻断钙电流的程度相似. **结论**: 蝙蝠葛碱具有阻断 L 型钙电流的作用.